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Effects of Growth Substances on the Absorption and Transport of Iron in Plants

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ABSTRACT

The effects of a number of growth substances on the absorption and translocation of iron were studied in bean plants. Gibberellic acid application to the trifoliate leaf enhanced absorption of Fe applied to the primary leaf. 2-Chloroethyltrimethylammonium chloride increased absorption by the primary leaf while 6-furfurylaminopurine (kinetin) increased the transport of Fe from the primary leaf to other parts. When the roots were pretreated with gibberellic acid, the absorption of Fe by the primary leaf and subsequent transport to the trifoliate leaves were increased. Triiodobenzoic acid reduced the absorption and transport of Fe.

Absorption of Fe by roots and transport to other parts were increased by pretreatment of the roots with gibberellic acid, 2-chloroethyltrimethylammonium chloride or *N,N*-dimethylaminosuccinamic acid for 3 days. An increase in the transport to other parts of Fe absorbed by roots was obtained when roots were exposed to the growth substances following Fe absorption.

Absorption and transport of Fe in corn plants were much less than those of Rb and phosphate. Absorption and transport of Fe were greater in plants with decorticated roots than in those with intact roots. *N,N*-Dimethylaminosuccinamic acid significantly promoted the transport of root-absorbed Fe to the shoots of corn plants.

Probably the oldest scientific report on the correction of Fe chlorosis dates back to 1844 (6). Despite the fact that extensive research on the mechanism of Fe uptake and effective means of supplying Fe to plants has been documented (3, 4, 20, 21), Fe nutrition still remains one of the most challenging plant nutritional problems today. The use of synthetic chelates to supply Fe has been advocated (20), but this has limitations (21). Furthermore, Fe chelates are not absorbed as much as Fe salts although greater percentages of the former are translocated (9).

It has been shown that Fe absorption by roots and leaves is metabolic (11, 20). Growth substances are known to increase, retard, or modify plant growth by affecting diverse metabolic processes (18, 19), and it is likely that these substances also affect the mechanism of ion uptake (17). However, very little information relating to the action of growth substances on the absorption and transport of Fe is available in the literature (1, 12, 20). The present paper deals with the effects of 6-furfurylaminopurine, gibberellic acid, 2-chloroethyltrimethylammonium chloride, *N,N*-dimethylaminosuccinamic acid, 4-hydroxy-5-isopropyl-2-methylphenyl trimethylammonium chloride 1-piperidine carboxylate (Amo 1618), and triiodobenzoic acid on Fe absorption by leaves and roots, and its transport to other organs.

MATERIALS AND METHODS

Plant Materials. Bean (*Phaseolus vulgaris* L.) seeds were surface sterilized with 1% sodium hypochlorite and germinated in the dark, on a filter paper supported by a stainless steel screen which was kept in a tray containing aerated distilled water. After 3 days the seedlings were transferred to aerated half-strength Hoagland nutrient solution and grown under a 12-hr photoperiod regime (500 ft-c) at 25°. Maize (*Zea mays* L.) seeds were germinated in the dark on cheesecloth supported by a nylon screen over aerated distilled water containing 0.2 mM $CaSO_4$, and after 3 days they were placed under a 12-hr photoperiod. The maize seedlings were grown in aerated 0.2 mM $CaSO_4$ solution which was renewed once in 2 days. Seven- to 10-day-old plants were used in the experiments. All the experiments were carried out at room temperature (25 ± 1°).

Effects of Growth Substances on Foliar Absorption and Transport of Fe in Bean Plants. In one experiment, 0.2 ml of each of the growth substances (kinetin, CCC,[1] and Amo 1618 at 10^{-3}M; GA_3 and TIBA at 10^{-4}M) was applied by means of a nylon mesh disc (3.1 cm^2) which was fixed to the center of the terminal leaflet of the first trifoliate leaf. After 2 hr, 0.5 ml of ^{59}Fe-labeled 1 mM $FeSO_4$ (specific radioactivity 0.1 mc/mmole; pH 5.0) was supplied to one of the primary leaves by means of a nylon mesh disc. The plants were harvested after 2 days. Foliar absorption and transport of Fe were further studied by pretreating the roots with growth substances. Bean plants were transferred to aerated nutrient solutions (without Fe) containing growth substances (GA_3, 10^{-5}M; CCC, 5×10^{-4}M; kinetin, 10^{-4}M; Amo 1618, 10^{-4}M; or TIBA, 10^{-5}M). After 3 days, 0.1 ml of ^{59}Fe-labeled $FeSO_4$ solution was applied to one of the primary leaves. The plants were harvested 2 days later and radioassayed.

Effects of Growth Substances on Absorption of Fe by Roots and Its Translocation. Bean and maize seedlings were transferred to aerated nutrient solution (without Fe) containing GA_3, CCC, or Alar at 10^{-4}M each. After 3 days, one batch of bean seedlings was placed in beakers containing 60 ml of ^{59}Fe-labeled $FeSO_4$ for 4 hr, under 500 ft-c light. The influence of growth substances on the translocation of root-absorbed Fe within the plant was examined in another batch of seedlings which was first allowed to absorb Fe for 4 hr under light and then transferred to nutrient solution containing growth substances. The roots of some of the maize seedlings pretreated with growth substances were decorticated mechanically (2, 14). The plants with intact roots and decorticated roots were mounted on waxed filter paper discs (15) and kept in a beaker containing 200 ml of ^{59}Fe-labeled

[1] Abbreviations: kinetin: 6-furfurylaminopurine; GA_3: gibberellic acid; CCC: 2-chloroethyltrimethylammonium chloride; Alar: *N,N*-dimethylaminosuccinamic acid; TIBA: triiodobenzoic acid.

$FeSO_4$ under light for 3 hr. After appropriate intervals the plants were harvested and radioassayed.

Radioassay of Plant Materials. Tissues which were treated with labeled solutions were washed in 1 mM solutions of the respective nonlabeled salts for 15 min and then rinsed in deionized water, in order to remove the "free space" and adsorbed ions. The activity remaining in the tissue after desorption was taken to indicate the amount absorbed by the tissue. Radioactivity of the ashed samples was measured in a G.M. scaler. ^{14}C activity was assayed in a Packard liquid scintillation spectrometer.

Each experiment consisted of three to four replicates with three or five plants per replicate. The absorption and transport to different parts are expressed as absolute amounts of Fe per set of three bean or five corn plants.

RESULTS AND DISCUSSION

Absorption of Fe by the primary leaf was increased by treatment of the trifoliate leaf with CCC or GA_3 (Fig. 1). The uptake was increased significantly more by CCC than by GA_3. Transport of Fe to trifoliate leaves was enhanced by GA_3 and kinetin. Kinetin, CCC, and Amo 1618 increased the downward transport, *viz.*, to the stem and roots, significantly more than with control, while the effect of GA_3 was less significant.

Treatment of the roots with GA_3 or CCC increased Fe absorption by the primary leaf while the former enhanced the transport to trifoliate leaves also (Fig. 2). The influence of GA_3 on Fe absorption and transport may be related to its effect on plant metabolism in general. Similar effects of GA_3 on foliar absorption and transport of Rb have been reported (7). Absorption of

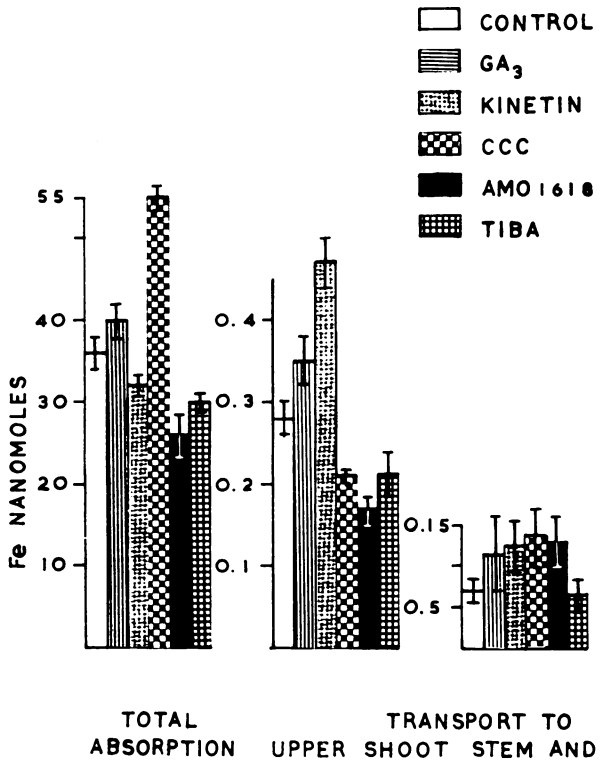

FIG. 1. Absorption of Fe by the primary leaf and transport to upper shoot (trifoliate leaves) and to stem and roots in bean plants. Growth substances (0.2 ml of kinetin, CCC, or Amo 1618 at 10^{-3}M; GA_3 or TIBA at 10^{-4}M) were applied to the terminal leaflets of the first trifoliate leaves, and ^{59}Fe-labeled $FeSO_4$ was supplied to one of the primary leaves. Absorption and transport were measured after 2 days. The vertical bars are the standard deviations of the means.

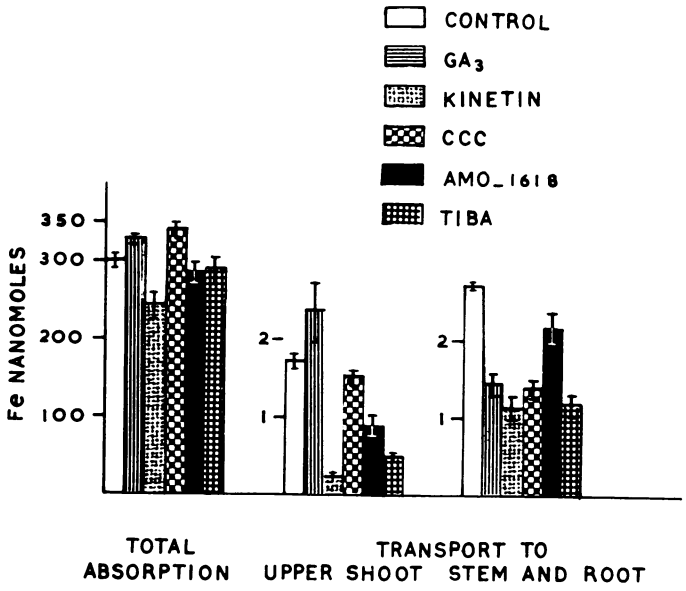

FIG. 2. Absorption of Fe by the primary leaf and transport to upper shoot and to stem and roots in bean plants which were pretreated with growth substances (GA_3, 10^{-5}M; CCC, 5×10^{-4}M; kinetin, 10^{-4}M; Amo 1618, 10^{-4}M; or TIBA, 10^{-5}M) in nutrient solution for 3 days. ^{59}Fe-Labeled $FeSO_4$ was supplied to one of the primary leaves. Absorption and transport were measured 2 days later. The vertical bars are the standard deviations of the means.

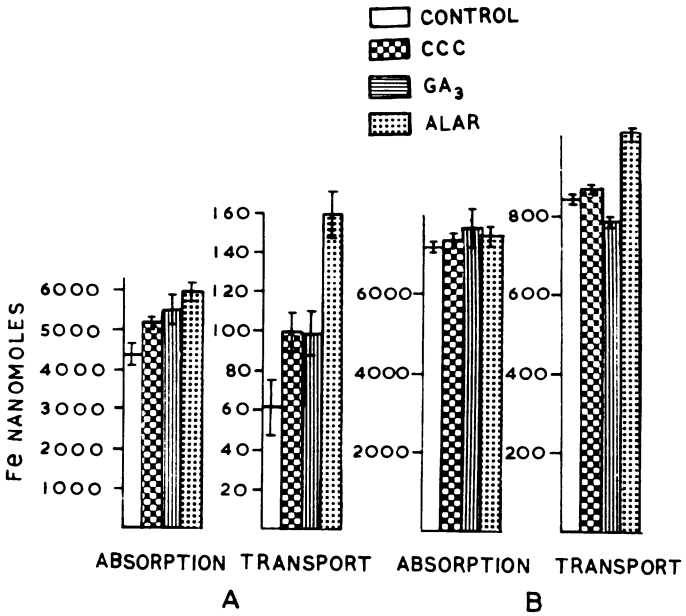

FIG. 3. Effects of GA_3, CCC, or Alar at 10^{-4}M each, on the absorption of Fe by roots and translocation. A: The bean plants were kept in nutrient solution containing growth substances for 3 days, and absorption and transport of Fe were measured after exposing the roots to ^{59}Fe-labeled $FeSO_4$ for 4 hr under 500 ft-c light. B: The bean plants were first exposed to ^{59}Fe-labeled $FeSO_4$ for 4 hr under the light and then transferred to nutrient solution containing growth substances. Absorption and transport to parts other than roots were measured after 3 days.

a nutrient ion and its subsequent transport to other parts are not necessarily related to each other. This is especially true with Fe, which is translocated less in relation to its absorption (9, 20). The growth substances included in the experiments differ in

their effects on the absorption and transport of root- and foliar-applied Fe. The treatment of roots with TIBA reduced the transport of Fe from the primary leaf to upper shoot and also to the stem and root significantly (Fig. 2), and foliar-applied TIBA reduced the transport to upper shoot (Fig. 1). Although Kessler and Moscicki (12) found TIBA to increase the translocation and utilization of foliar-applied Fe, the studies of Bar-Akiva and Hewitt (1) did not support the findings. With regard to kinetin, there is a general tendency to mobilize Fe toward kinetin-treated parts. This is in agreement with the observations by Müller and Leopold (17) on the kinetin-induced "directed transport" of Na and phosphate.

One criterion of the effectiveness of the supply of Fe to foliage is the extent of its translocation to other parts from the site of application, and therefore the effects of growth substances on Fe translocation are of greater significance than those on Fe absorption. Furthermore, the amount of Fe retained in the treated leaf would include a significant fraction due to adsorption, despite the washing and desorption of the tissue at the end of the experiment. Root-absorbed GA_3 (Fig. 2) and foliar-absorbed GA_3 and kinetin are the only treatments which have significantly enhanced the translocation to the upper shoot.

The roots of bean plants pretreated with CCC, GA_3, or Alar absorbed and translocated more Fe than the control (Fig. 3). A significant enhancement of translocation of Fe was also observed when roots were exposed to Alar subsequent to Fe absorption. The results further suggest that CCC and GA_3 would not influence the transport unless the roots were pretreated with these chemicals (Fig. 3A).

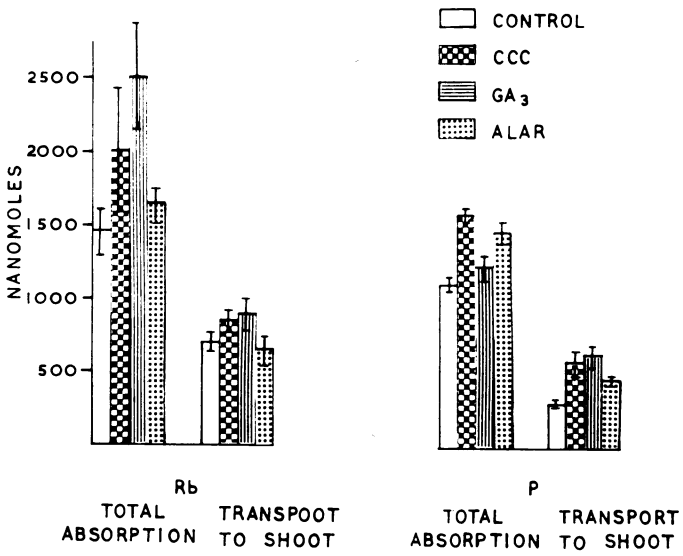

FIG. 4. Absorption and transport of Rb and phosphate in maize plants pretreated with GA_3, CCC, or Alar at 10^{-4}M each for 3 days and then exposed to ^{86}Rb-labeled RbCl or ^{32}P-labeled H_3PO_4 for 3 hr under the light.

Table I. *Absorption of Fe by Excised Bean Roots from ^{59}Fe-labeled 1 mM $FeSO_4$ Solution in Absence and Presence of Growth Substances and Metabolic Inhibitors*

The experimental solution (1 liter) contained 0.2 mM $CaSO_4$ and other chemicals.

Treatment	Fe Absorption in Roots		
	No inhibitor	DNP (10^{-4}M)	NaN_3 (10^{-4}M)
	nmoles/100 mg · 2 hrs		
Control	441 ± 22.4	310 ± 20.0	320 ± 12.0
GA_3, 10^{-4}M	451 ± 23.2	261 ± 16.8	285 ± 12.8
CCC, 10^{-4}M	456 ± 8.8	268 ± 6.4	273 ± 8.8
Alar, 10^{-4}M	336 ± 20.0	178 ± 16.8	238 ± 7.2
TIBA, 10^{-4}M	248 ± 21.6	256 ± 2.4	246 ± 17.6

Table II. *Uptake and Incorporation of ^{14}C-Uracil and ^{14}C-Glycine into RNA and Protein, Respectively, in Excised Roots of Bean Plants Pretreated with Growth Substances (10^{-4}M) for 3 days*

Roots (100 mg) were incubated in 20 ml of 1 mM labeled solutions for 6 hr, and radioactivity was measured in 70% ethanol-soluble and -insoluble portions.

Treatment	^{14}C-Uracil			^{14}C-Glycine		
	Total uptake	Incorporation	Incorporation	Total uptake	Incorporation	Incorporation
	cpm × 10^{-2}		%	*cpm* × 10^{-2}		%
Control	142 ± 8	68 ± 7	47.9	4851 ± 672	2371 ± 251	48.8
CCC	176 ± 18	67 ± 8	38.1	3006 ± 117	1720 ± 166	57.2
GA_3	259 ± 6	131 ± 1	50.5	2161 ± 46	1578 ± 11	73.0
Alar	92 ± 16	60 ± 5	65.2	5783 ± 17	3137 ± 405	54.2

The influence of the growth substances on the absorption and transport of other ions, *viz.*, Rb and phosphate, was examined by pretreatment of maize seedlings with growth substances for 3 days and subsequent exposure to ^{86}Rb-labeled RbCl or ^{32}P-labeled H_3PO_4 (conc 1 mM; specific radioactivity 0.1 mc/mmole) for 3 hr under 500 ft-c light. CCC and GA_3 increased the absorption of Rb and CCC and Alar enhanced phosphate uptake significantly (Fig. 4). CCC and GA_3 increased the transport of both Rb and phosphate.

The growth substances used in the experiments include chemicals which are known to promote growth and those which retard growth. CCC and Alar (growth retardants) increased Fe absorption to more or less the same extent as GA_3 (Fig. 3A) while Alar was much more effective than GA_3 and CCC on Fe translocation. The mechanism by which these chemicals of diverse nature act on the ion uptake process is not known. A possible explanation would lie in the "source and sink" theory (17), and the actively growing shoot region is assumed to be the "sink" area for ions. The growth promoters and retardants might exert their influence on the actively growing regions and help mobilize the nutrients absorbed elsewhere toward the shoot. Further studies with these chemicals on similar lines as reported for kinetin (17) would perhaps be more revealing.

Ion absorption by plants is a complex process, and a number of factors affecting the process have been recognized. Since phosphorylation (8) and protein synthesis (5) are involved in ion uptake, it seemed worthwhile to study the effect of the interaction of growth substances and inhibitors. Excised roots (100 mg) of bean plants were suspended in medium (1 liter) containing ^{59}Fe-labeled 1 mM $FeSO_4$, 0.2 mM $CaSO_4$, and growth substances. Fe absorption was measured for 3 hr in the absence and presence of DNP or NaN_3 (10^{-4} M). Absorption was reduced by the inhibitors even in the presence of growth substances (Table I). DNP reduced Fe uptake significantly in the presence of Alar. There was no effect of inhibitors in the presence of TIBA although TIBA itself reduced Fe uptake drastically.

In order to test the influence of CCC, GA_3, and Alar on RNA and protein synthesis, excised roots from bean plants pretreated with these chemicals were incubated in 20 ml of 1 mM ^{14}C-labeled glycine or uracil (specific radioactivity 10 mc/mmole) for 6 hr, and the radioactivity in RNA and protein fractions (13) was

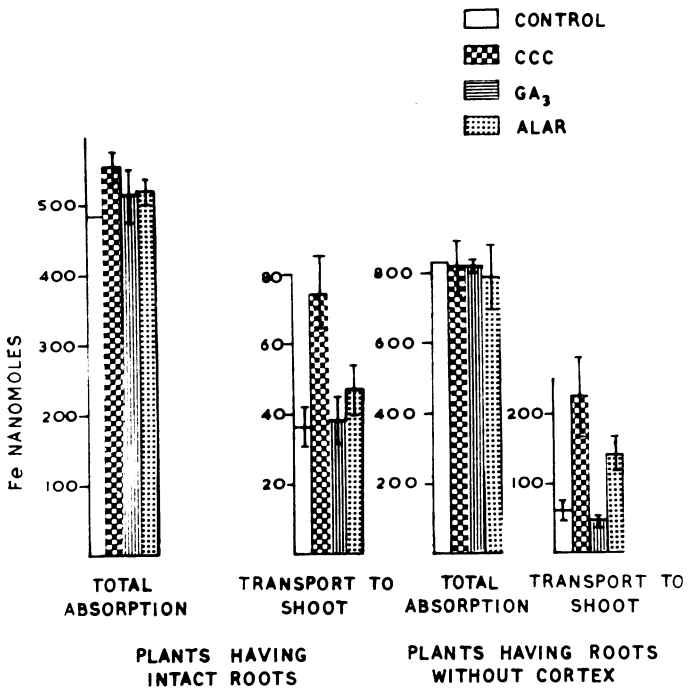

FIG. 5. Absorption and transport of Fe in maize plants pretreated with GA_3, CCC, or Alar at 10^{-4}M each, after which the roots of one set of plants were decorticated. Plants with intact roots and with decorticated roots were exposed to ^{59}Fe-labeled $FeSO_4$ for 3 hr under the light, and the absorption and transport were measured.

measured (Table II). Alar increased the total uptake of ^{14}C-glycine and also incorporation into protein. CCC and GA_3 reduced both uptake and incorporation into protein. Percentage incorporation of ^{14}C-glycine and that of ^{14}C-uracil were increased significantly by GA_3 and Alar, respectively. These results suggest that the general reaction of the plant is affected by growth substances, and the resultant increase in metabolism in various "sinks" such as the trifoliate leaves and growing points in intact plants leads to increased translocation accompanied by increased absorption. Another possibility would be that the growth substances influence membrane permeability and increase the rates of ion entry through the membrane.

Fe is generally considered an immobile element in plants (4). However, it has been reported that foliar-applied Fe is fairly mobile in sorghum, cotton, and bean (3). Evidence presented (Figs. 4 and 5) shows that Fe is poorly translocated in maize plants. Absorption and percentage translocation of Fe are very much less than those for Rb and phosphate. The transport of Fe to shoot in plants having intact roots and those with decorticated roots is enhanced more significantly by CCC and less so by Alar, when compared to their respective controls. It is interesting that Fe absorption and translocation are significantly greater in plants with decorticated roots than in those with intact roots. This is in contrast to the findings of Lüttge and Laties (16) that the stele is less efficient than the cortex in ion accumulation. It has been shown recently that the stele of 6-day-old corn seedlings could accumulate salt as vigorously as the cortex (22).

Kinetics of Fe absorption by enzymically isolated cells have revealed that Fe has a low affinity constant (10), and this implies that the hypothetical carrier for Fe binds Fe less efficiently. The poor absorption of Fe by root cortex is probably another causative factor for Fe chlorosis, and a detailed study of the kinetics of Fe absorption would be quite revealing. Chemicals that could enhance this affinity between ions and ion carriers should further help in solving problems of Fe nutrition.

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